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Comparison of artificial sebum with human and hamster sebum samples

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ABSTRACT

To understand drug delivery to the sebum filled hair and sebaceous follicles, it is essential to use an artificial sebum as a surrogate of the human sebum for the investigation of drug transport properties. Artificial sebum L was developed in-house based on the chemical similarity to human sebum. The partition and diffusion of model compounds (ethyl 4-hydroxybenzoate, butyl 4-hydroxybenzoate, and hexyl 4-hydroxybenzoate) were measured in human sebum, hamster ear and body sebum (a commonly used animal model), and four representative artificial sebum samples (N, S, F, and L) in which artificial sebums, N, S and F were selected based on the available literature. DSC and NMR studies were also conducted on all sebums to compare their melting properties and chemical compositions. *In vitro* studies show that the partition coefficients of the three model compounds in artificial sebum L were similar to that of human sebum, whereas the hamster ear and body sebum, and other three artificial sebum samples were different from that of human sebum. Additionally, the *in vitro* sebum flux ($\mu g/(cm^2\ min)$) of three model compounds through artificial sebum L was closer to that of human sebum when compared with the other three artificial sebum (N, S and F), hamster body and hamster ear sebum. The results of this study indicate that the artificial sebum L could be used as an alternative to human sebum, as the physicochemical properties of this artificial sebum is relatively similar to human sebum.

1. Introduction

Traditionally, the transepidermal route has been recognized as the primary pathway for topically applied therapeutic agents, despite the fact that the stratum corneum functions more as a rate-limiting barrier and less as a pathway for the absorption of topically applied compounds (Lauer et al., 1995; Williams and Elias, 1987; Scheuplein, 1967). However, transfollicular delivery of topically applied agents has been increasingly recognized as an efficient approach to enhance regional efficacy and reduce systemic exposure (Bertolino et al., 1993; Grams et al., 2004; Lademann et al., 2001). While it is estimated that hair and sebaceous follicles cover only about 0.1% of the total skin area of the body, this fraction rises to as much as 10% in the scalp and facial regions, making transfollicular delivery into and/or through these regions very significant (Schaefer and Redelmeier, 1996). Further, the barrier function through the follicular surface is substantially lower due to the lack of multiple-layer, tightly integrated corneocytes (as found in the stratum corneum). Transfollicular delivery of topical agents is possible as long as the drug permeation into follicles is faster than the outflow of sebum from sebaceous glands to the skin surface (Agarwal et al., 2000).

Since the upper sections of hair and sebaceous follicles are filled with sebum, the efficiency of follicular drug delivery substantially depends on the partition/diffusion of the drug molecules in the sebum. Partition/diffusion properties of drug molecules in human sebum have not been investigated due to limitations in collecting sebum samples from human subjects. Hence, it is desirable to develop an appropriate artificial sebum as an alternative to human sebum for the investigation of follicular drug delivery. There are about 18 artificial sebum recipes reported in the literature (Stefaniak and Harvey, 2006) and the composition of the reported artificial sebum samples varied significantly. The partition and diffusion properties of topical molecules in these artificial sebum and human sebum samples have not been investigated. Having compared the chemical compositions of these artificial sebum samples with human sebum, we formulated an artificial sebum (L) which has closer chemical composition and so physicochemical properties to human sebum, and determined the melting points, NMR profiles and the partition/diffusion properties of several model compounds to ensure the usefulness of the newly developed artificial sebum. Artificial sebum L has been used as a tool for screening potential

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sebum-targeted drug molecules (Valiveti et al., 2008; Valiveti and Lu, 2007). Since hamster ear models have been commonly accepted as an animal model for follicular drug delivery (Meidan et al., 2005), we also compared hamster sebum samples with human sebum and the artificial sebums to bridge various models.

2. Materials and methods

2.1. Materials

Ethyl 4-hydroxy benzoate and butyl 4-hydroxybenzoate from Lancaster synthesis, Inc. (Pelham, NH). Hexyl 4-hydroxybenzoate was obtained from TCI America (Portland, OR). Paraffin wax (melting point, 58–62 °C), and oleic acid were obtained from Aldrich Chemical Company, Inc. (Milwaukee, WI). Cottonseed oil, palmitoleic acid, squalene, palmitic acid myristyl ester, palmitic acid palmitic ester, oleic acid palmitic ester, stearic acid, oleic acid, and myristic acid were obtained from M.P. Biomedical, LLC (Aurora, OH). Coconut oil was obtained from Aldon Corporation (Avon, NY). Tripalmitin, tripalmitolein, and triolein were obtained from Sigma Chemical Company, Inc. (St. Louis, MO). Olive oil and palmitic acid were obtained from EMD Chemicals (Gibbstown, NJ). Spermaceti wax and cholesterol oleate were obtained from Sargent–Welch (Buffalo, IL) and Tokyo Kasei Kogyo Co., Ltd. (Tokyo, Japan) respectively. Acetonitrile, trimethylpentane, ethyl acetate, water for HPLC and acetic acid were obtained from Millinckrodt Baker, Inc. (Philipsburg, NJ).

2.2. Instruments

Equipment used consisted of a 1100 series high-pressure liquid chromatography (HPLC) instrument with a Agilent 1100 series autosampler and a Diode Array detector model 785A (Agilent Technologies, Inc., Palo Alto, CA), an Innova® 4000 series incubator and shaker (New Brunswick Scientific Co., Inc, Edison, NJ), Hermle Z360K centrifuge (Maschinenfabrik Berthold Hermle, Gosheim, Germany), a Q1000 differential scanning calorimeter (DSC) (TA instruments, New Castle, DE), and an 600 MHz NMR spectrometer (Varian, Inc., Palo Alto, CA).

2.3. HPLC conditions

Samples from *in vitro* partition and diffusion experiments were analyzed by HPLC. A reversed phase 150 mm × 4.6 mm symmetry®-C_{18} 3.5-μm column was used and the wavelength used was 254 nm. The mobile phase for three model drugs consisted of 1% acetic acid and acetonitrile (20:80) at a flow rate of 2 mL/min. The injection volume was 50 μL and the run time was 2.5 min. The retention times of ethyl 4-hydroxybenzoate, butyl 4-hydroxybenzoate and hexyl 4-hydroxybenzoate were 0.90, 1.1 and 1.6 min, respectively.

2.4. Preparation of human sebum and hamster sebum samples

2.4.1. Extraction of human sebum from Sebutape®

Human sebum samples were collected using Sebutapes® skin indicators which were provided by the clinical study group, Pfizer Inc., Ann arbor, MI. Sebutape® skin indicators are non-adhesive sebum collecting devices incorporating a dark background for quickly estimating surface sebum. The Sebutape® indicators function through the gradual displacement of air in the micro-porous film by sebum, then change in appearance revealing a black background indicating the location of the active oil glands. The size of the transparent area (black) is a measure of the amount of sebum collected. Approximately 500 sebum-enriched Sebutapes® were extracted with 50 mL of cyclohexane in a 100 mL clean glass bottle for 30 min at 425 rpm in a shaker at 37 °C. Then, the supernatant liquid was evaporated under nitrogen at 40 °C until constant weight was achieved. These samples were used for DSC, NMR, partition coefficient and diffusion studies.

2.4.2. Extraction of hamster ear and body sebum

All studies were approved by the Institutional Animal Care and Use Committee. Male Syrian hamsters (age 9–10 weeks) were purchased from Harlan (Indianapolis, IN). The animals were acclimated 2 weeks to a 14-h light cycle and received a standard rodent diet (Labdiet, Richmond, IN) and water ad libitum. The animals were sacrificed by asphyxiation using carbon dioxide. The ears were excised for the extraction of ear sebum lipids and the hamster body was used for the extraction of body sebum lipids. For the extraction of ear sebum lipids, the base of the ears were clipped to a suspension bar and lowered into 3 mL of extraction solvent containing trimethylpentane and ethyl acetate (4:1) in a glass 1-dram vial until the base of the ear was 2 mm above the solvent surface for 30 min. For the extraction of hamster body sebum lipids, the entire body was immersed in the extraction solvent for 30 min. After then, 1 mL of water was added to the lipid extraction sample, vortexed for 5 min and centrifuged for 5 min at 1600 rpm. Two mL of the organic layer was transferred to a clean 1-dram glass vial. The sample was then dried down under a stream of nitrogen and lyophilized overnight. The dried hamster ear and body sebum were used for the studies.

2.5. Preparation of artificial sebum

The lipids from the skin surface are derived mainly from two sources, the sebaceous glands and the epidermis (Strauss et al., 1976). The surface lipids from the gland-rich areas naturally contain a higher proportion of sebaceous lipid. In gland deficient areas such as the arms and legs there is a greater proportion of epidermally derived lipid whereas the skin lipids from the face are derived in large part from the sebum. The main components of the sebum are triglycerides, wax esters, squalene, cholesterol and cholesterol esters (Strauss et al., 1976). The chemical composition of artificial sebum L was based on the human sebum chemical compositions reported in the literature (Wertz, 2002; Rosenthal, 1964; Greene et al., 1970; Nordstrom et al., 1986; Stewart et al., 1978), which are illustrated in Table 1. The components of artificial sebum were obtained from commercially available sources (Table 2). The ingredients were weighed out (% w/w) in a glass beaker and were heated at 60 °C with intermittent stirring until all the solids became a clear liquid (10 min). This was done to ensure uniform mixing of the model sebum lipids. The mixture was allowed to cool down at room temperature and was used for the studies. All components of the artificial sebum are miscible at 60 °C and there were no visual indications of separation of sebum lipids. Moreover, the variation in the *in vitro* partition and diffusion data from different lots of the artificial sebum was less than 20% indicating mixing of the sebum components is homogenous. Artificial sebum N, S and F (Table 2) were prepared based on the composition given in the literature (Stewart et al., 1978; Spangler et al., 1967; Friberg and Osborne, 1986).

2.6. Proton NMR method

NMR spectra were acquired with a 600 MHz Varian Inova NMR spectrometer running VNMRJ software version 1.1C, equipped with a 5 mm triple resonance cryoprobe. The acquisition parameters included a 15 ppm spectral width, 85° pulse, 1.8-s acquisition time, 1-s relaxation delay, 32 K data points, and 512 scans. All data were acquired at 298 K. Data were processed

Table 1
Chemical composition of human sebum.

Composition	Human sebum-I[a] (lumen/surface)	Human sebum-II[b]	Human sebum-III[c]	Human sebum-IV[d]	Human sebum-V[e]
Squalene	15/15	13	12	19.9	13.6
Wax esters	25/25	26	26	25.3	19.0
Triglycerides	57/42	32	57.5	16.1	63.6
Fatty acids	0/15 (C16)	23	–	33.0	1.2
Cholesterol	1/1	1.6	1.5	3.8	0.6
Cholesteryl esters	2/2	3.5	2.0	2.0	2.0

[a] Human sebum-I: Wertz (2002).
[b] Human sebum-II: Rosenthal (1964).
[c] Human sebum-III: Greene et al. (1970).
[d] Human sebum-IV (acne): Nordstrom et al. (1986).
[e] Human sebum-V: Stewart et al. (1978).

using the in-house software, metabonomic, written by J. David Baker described in WO2004038602 (patent pending). Processing included a Fourier transform, phasing of the spectrum, and baseline correction. Spectra were referenced to the low levels of protonated cyclohexane in the sample at 1.38 ppm and standardized to 32 K points in the spectra region from −0.5 to 10 ppm. Spectral regions containing each analyte were integrated including 5.04–5.13 ppm for squalene, 4.52–4.60 ppm for cholesterol esters, 4.20–4.30 ppm for triglycerides, and 3.96–3.99 for wax esters.

2.7. DSC studies

DSC scans were run using a ramp rate of 5 °C/min from −30 to 80 °C. 1–5 mg of sample was weighed into a tared (pre-weighed) DSC pan. DSC pan weight was taken into account in the TA Instruments software. Samples were covered with a pin-hole punched lid. DSC pans were not crimped, since the sebum tended to leak out of the pin-hole. DSC scans were evaluated for any transitions, including a melting endotherm. However, since the sebum mixtures are quite complex, a sharp and clear melting endotherm was not readily observed. Thus, a range is given for the melting points of the different sebum samples.

Table 2
Composition of artificial sebum.

Component	Composition of artificial sebum (% w/w)			
	N[a]	S[b]	F[c]	L[d]
Squalene	13		15	15
Wax esters				
Paraffin wax		15		10
Spermaceti				15
Palmitic acid myristyl ester	27			
Palmitic acid palmitic ester			10	
Oleic acid palmitic ester			10	
Triglycerides				
Olive oil		20		10
Coconut oil		15		10
Cottonseed oil				25
Tripalmitin	6.67		20	
Tripalmitolein	3.33			
Triolein			20	
Fatty acids				
Stearic acid		15		
Oleic acid		15	6	1.4
Palmitoleic acid	16.67			5
Palmitic acid	33.33		10	5
Myristic acid			4	
Cholesterol		20	3	1.2
Cholesterol esters				
Cholesterol oleate			1	2.4
Cholesteryl palmitate			1	

[a] Artificial sebum N: Nordstrom et al. (1986).
[b] Artificial sebum S: Spangler et al. (1967).
[c] Artificial sebum F: Friberg and Osborne (1986).
[d] Artificial sebum L: developed in the authors' lab.

2.8. Determination of sebum partition coefficient

Sebum partition coefficient studies were conducted in a 2-mL glass vial with a weighed amount (1–20 mg) of artificial sebum, hamster sebum or human sebum sample. The sebum samples in the vials were equilibrated with 1 mL of aqueous drug solution (~20 μg/mL of a model compound in citrate–phosphate buffer, pH 5.5) in a shaker for 15 h at 37 °C. The mixed samples were then centrifuged at 8000 rpm for 15 min and the clear aqueous solution was withdrawn and analyzed by HPLC for drug content. The amount of drug partitioned into the sebum samples was calculated by subtracting the amount of drug concentration present in the aqueous solution from the initial concentration of the aqueous drug solution. The partition coefficient values were expressed as the concentration of drug in 1 g of sebum divided by the concentration of drug in 1 g of aqueous solution.

2.9. Drug transport through the artificial, hamster, and human sebum

The drug transport through the artificial sebum was carried out in 24-well format (Transwell®, Corning Incorporated, NY). The supporting membrane (polycarbonate membrane, 0.45 μm thickness) of each insert was coated with 2.1 ± 0.2 mg of the artificial sebum (previously heated at 50–55 °C). A 150-μL aliquot of aqueous suspension of model drug (10 mg/mL in citrate–phosphate buffer (CPB, pH 5.5)) was loaded onto the insert and 1 mL of preheated (37 °C) 10% HP-β-cyclodextrin in the CPB was used as receiver solution. The entire study was carried out in an incubator at 37 °C and 125 rpm. The sampling interval was every 10 min for 2 h. At each sampling time, the entire receiver solution was replaced with fresh buffer. The withdrawn samples were analyzed for drug content by HPLC. The cumulative quantity of drug collected in the receiver compartment was plotted as a function of time. The flux value for a given experiment was obtained from the slope (steady-state portion) of the cumulative amount of drug permeated vs. time plot. The aqueous solubility of each compound was determined by centrifugation of the suspension used for the donor phase and analysis of the supernatant with HPLC. The permeability coefficients were calculated from the steady state flux and the drug concentration (solubility) in the vehicle. The diffusion coefficients were calculated using permeability coefficients, the thickness of sebum layer, and the sebum partition coefficient.

2.10. Statistical analysis

Statistical comparisons were made using one-way analysis of variance and Turkey post hoc test with the help of using SigmaStat (SPSS, Inc., Chicago, IL). A value of $P < 0.05$ was considered statistically significant.

3. Results and discussion

Human sebum constantly secretes from sebaceous glands onto the skin surface, especially on the facial and scalp skin areas. Therefore, sebum acts as the first layer of transport medium to a topically applied cosmetic or pharmaceutical compound. Due to its high lipophilicity, sebum functions as a permeation barrier to hydrophilic compounds while serving as a good solvent for lipophilic compounds. The interaction of sebum with topical formulation significantly affects both the barrier function of sebum as well as the spreading property of the formulation on the skin, and therefore affects the deliverability of an active ingredient from the formulation into or through the skin.

As shown in Table 2, artificial sebum L developed in our lab was used for our previous and present studies (Valiveti et al., 2008; Valiveti and Lu, 2007). The chemical composition (Table 2) of artificial sebum L is similar to the chemical composition of human sebum (Table 1), whereas the composition of other artificial sebum samples N, S, and F are significantly different from human sebum. In the artificial sebum preparations, paraffin wax and spermaceti wax were the source of wax esters. Olive oil, cottonseed oil and coconut oil served as the triglycerides in artificial sebum L based on the similar carbon chain length of the fatty esters with the chemical compositions of human sebum. For further investigation, palm oil could be a good alternative source for the triglycerides in artificial sebum because of its high content of C_{16} fraction. The percentage of free fatty acids in human sebum samples varies greatly and depends on the degree of triglycerides degradation during sebum secretion from the sebaceous glands to the skin surface. According to Wertz's analysis, the concentration of free fatty acid was undetectable in the lumen and 15% in the surface (Wertz, 2002). Other studies reported 23, 0, 33 and 1.2% fatty acids respectively (Rosenthal, 1964; Greene et al., 1970; Nordstrom et al., 1986; Stewart et al., 1978). Interestingly, the total content of triglycerides and fatty acids in human sebum from the cited studies were 57, 55, 57.5, 48.1 and 63.6% respectively, which is relatively consistent (Wertz, 2002; Rosenthal, 1964; Greene et al., 1970; Nordstrom et al., 1986; Stewart et al., 1978). Considering the sebum of interest is in the upper duct of hair follicles, approximately 10% of free fatty acids and 55% of total triglycerides and fatty acids were formulated in our artificial sebum. Unlike human sebum, hamster ear and body sebum (Table 3) lacks squalene and contains a higher percent of wax esters (Brind et al., 1986) which could result in substantially different physicochemical properties from either human sebum or artificial sebum. To evaluate the usefulness of artificial sebum, the thermal behavior of the sebum sample, the drug partition in the sebum, and the diffusion properties through the sebum are considered as the most critical parameters. The present study was carried out to compare the partition/diffusion properties of artificial sebum samples with human and hamster sebum samples.

Table 3
Composition of hamster ear sebum.

Component	% w/w
Squalene	–
Sterol and wax esters	68
Triglycerides	20
Diol diesters	–
Fatty acids	5.5
Free sterols	2.9

Table 4
Melting points of the different sebum.

Sebum	T_m (onset; peak) in °C
Human sebum	32.9; 42.0
Artificial sebum-L	33.3; 40.28
Artificial sebum-N	24.2; 34.6
Artificial sebum-S	27.6; 34.1
Artificial sebum-F	7.9; 15.2
Hamster body sebum	33.1; 39.3
Hamster ear sebum	27.9; 35.0

3.1. Proton NMR method

Proton NMR spectroscopy was used to determine the composition profiles of sebum lipids in artificial, hamster and human sebum samples. Robosky et al. reported that the NMR spectroscopic method enabled the simultaneous and selective quantification of sebum lipids. It is simple, rapid, sensitive analytical method with wide linear dynamic range, especially useful for a small quantity of the samples (Robosky et al., 2008). The representative NMR spectra of the artificial sebum L, the hamster body sebum and the human sebum were given in Fig. 1. It is clear that the NMR spectrum of artificial sebum L is close to that of human sebum, whereas the NMR spectrum of hamster sebum is substantially diverse from human sebum, due to the absence of squalene and presence of higher amount of triglycerides in hamster sebum. This was in agreement with Brind et al.'s study in which the composition of hamster ear sebum was analyzed and characterized (Brind et al., 1986).

3.2. DSC studies

As mentioned above, sebum mixtures are quite complex and contain waxy materials, resulting in the endotherms lacking sharp melting peaks. Thus, a range is given for the melting points of the different sebum samples. As shown in Table 4, two major transitions were observed for all sebum samples. These thermal events may be due to the presence of saturated fatty acids in the artificial sebum (Motwani et al., 2002). Since there are no data available in the literature on the endotherms for human and hamster sebum samples, the comparison of the melting property is only relative to the tested samples. The transition temperatures of artificial sebum L (33.3 and 40.28 °C) are closer to human sebum transition temperatures (32.9 and 42 °C) when compared with other artificial sebum samples (N, S, and F). Artificial sebum F is in liquid state at room temperature hence it has lower transition temperatures (7.9 and 15.2 °C) when compared with other artificial, hamster and human sebum. The melting point transition of hamster ear sebum is lower than human sebum and artificial sebum L, where that of hamster body sebum is closer to human and artificial sebum L. This may be due to the presence of non-sebum lipids in the hamster body sebum samples by using the current extraction procedures. Based on the results, the melting point transitions of artificial sebum L are most desirable for a human sebum substitute.

3.3. Comparison of partition properties of artificial sebum samples with human and hamster sebum samples

The partition coefficient values of three model compounds in various artificial sebum (N, S, F and L), human sebum, hamster body sebum and hamster ear sebum were given in Table 5. The resulting partition coefficients (K_s) of three model compounds indicate

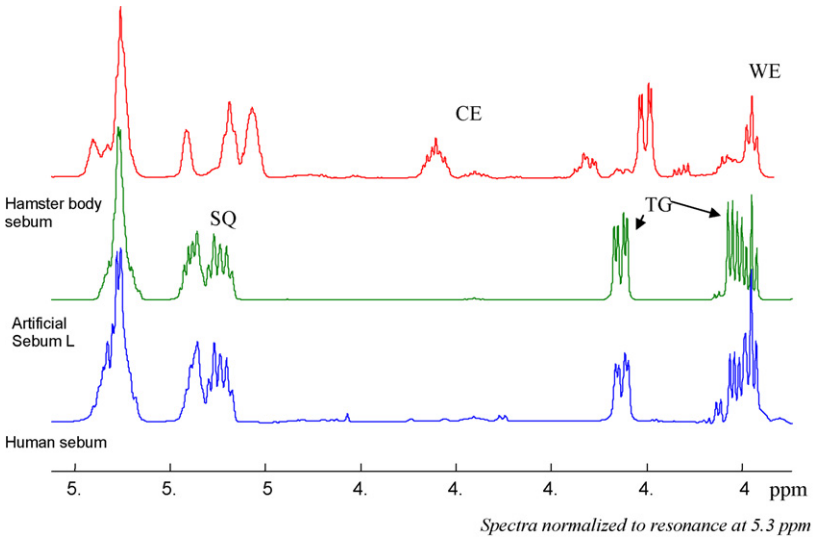

Fig. 1. NMR profiles of the sebum samples.

that among all the artificial sebum samples tested (N, S, F and L), only artificial sebum L showed no significant difference ($P > 0.05$) in K_s when compared with that of human sebum, demonstrating the usefulness and superiority of the artificial sebum developed in our lab compared to previously used artificial sebum preparations. Interestingly, the slopes of $\log K_s$ vs. carbon chain length of the model compounds for artificial sebum N, S and F varied from those for human sebum and artificial sebum L, suggesting a substantial difference among the artificial sebum samples. Despite similarity of hamster ear sebum to its body sebum in the partition coefficient values of model compounds, it is obvious that the partition properties of the model compounds in hamster sebum samples are significantly different from those of human sebum and artificial sebum L. Partition coefficient is one of the critical factors governing drug transport across sebum, so the deviation of drug partition in hamster sebum from human sebum should be taken into consideration when the hamster model is used. The results of the partition studies indicate that drug partition is sensitive to the composition of sebum, and artificial sebum L appears to be the preferable model for human sebum among various artificial sebum preparations because of the similar chemical composition to human sebum.

3.4. Comparison of diffusion properties of artificial sebum with human and hamster sebum samples

As described in the method section, the diffusion of the model compounds through sebum samples is carried out in 24-well apparatus. The effective permeation area and the thickness of the artificial sebum membrane loaded on the filter were calculated for the estimation of sebum flux and diffusion coefficient (Valiveti and Lu, 2007). Table 6 shows measured sebum flux (J_s) and aqueous solubility, as well as the calculated permeability coefficient (P_s) and sebum diffusion coefficient (D_s) of ethyl 4-hydroxybenzoate, butyl 4-hydroxybenzoate and hexyl 4-hydroxybenzoate in the artificial sebum samples, human sebum and hamster sebum (body and ear) samples. The variation of sebum flux within the same samples is generally less than 15% ($n = 6$). The diffusion study results of ethyl 4-hydroxybenzoate indicate that among the four artificial sebum samples, hamster ear and hamster body sebum samples, artificial sebum L showed no significant difference ($P < 0.05$) in the J_{ss}, P_s and D_s when compared with those of human sebum. However, there is a significant difference ($P > 0.05$) in the permeability properties (J_{ss}, P_s and D_s) of butyl 4-hydroxybenzoate through the artificial sebum, hamster ear and hamster body sebum when compared with those of human sebum, though artificial sebum L is the closest to the human sebum. In the case of hexyl 4-hydroxybenzoate (Table 6), artificial sebum N showed no significant difference ($P < 0.05$) in J_{ss}, P_s and D_s values when compared with human sebum but the other sebum samples were significant different ($P > 0.05$) from the human sebum. The ratios of the model sebum vs. human sebum in terms of J_{ss} are 1.15 (sebum L), 1.03 (sebum N), 0.016 (sebum S), 2.11 (sebum F), 0.029 (hamster body sebum), and 0.455 (hamster ear sebum), respectively. Although the sebum flux of hexyl 4-hydroxybenzoate in artificial sebum L was 15% faster then that in human sebum, this difference is within a reasonably acceptable margin. It is interesting to note that the transport of the model compounds in hamster ear sebum was slower than those in human sebum but the fluxes in hamster body sebum was substantially low (Table 6). Despite the similar partition behaviors of the

Table 5
The sebum/water partition coefficient of the tested compounds.

Sebum	Sebum/buffer (pH5.5) partition coefficient (K_s) at 37 °C (mean ± S.D.)			Linear relationship of $\log K_s$ vs. carbon chain length	
	Ethyl 4-hydroxybenzoate	Butyl 4-hydroxybenzoate	Hexyl 4-hydroxybenzoate	Slope	R^2
Human sebum	21.72 ± 0.61	276.39 ± 25.14	3233.96 ± 419.95	0.5425	0.9999
Artificial sebum-L	21.64 ± 0.13	285.11 ± 12.27	3229.23 ± 189.02	0.5437	0.9997
Artificial sebum-N	18.88 ± 2.46	341.32 ± 16.58	4561.52 ± 237.28	0.5957	0.999
Artificial sebum-S	34.97 ± 1.65	200.63 ± 13.11	1182.07 ± 193.54	0.3821	1.00
Artificial sebum-F	25.25 ± 0.62	352.2 ± 10.32	5071.76 ± 129.56	0.5755	1.00
Hamster body sebum	29.15 ± 0.08	375.52 ± 24.80	2656.17 ± 98.62	0.4899	0.9942
Hamster ear sebum	26.16 ± 2.06	364.14 ± 19.79	2525.77 ± 178.25	0.4962	0.9923

Table 6
Permeability parameters for the model compounds in artificial sebum, human sebum and hamster sebum ($n = 4$).

Sebum	Ethyl 4-hydroxybenzoate			Butyl 4-hydroxybenzoate			Hexyl 4-hydroxybenzoate		
	J_{ss}[a] (μg/(cm² min))	K_p[b] ($\times 10^{-3}$ cm/s)	D[c] ($\times 10^{-8}$ cm²/s)	J_{ss}[a] (μg/(cm² min))	K_p[b] ($\times 10^{-3}$ cm/s)	D[c] ($\times 10^{-8}$ cm²/s)	J_{ss}[a] (μg/(cm² min))	K_p[b] ($\times 10^{-3}$ cm/s)	D[c] ($\times 10^{-8}$ cm²/s)
Human sebum	110.05 ± 5.96	2.97 ± 0.16	82.08 ± 4.45	223.08 ± 4.25	22.80 ± 0.43	50.21 ± 0.94	229.55 ± 4.56	272.38 ± 5.65	50.53 ± 1.05
Artificial sebum-L	101.52 ± 7.22	2.74 ± 0.2	76.00 ± 5.40	169.89 ± 7.26	17.36 ± 0.74	36.65 ± 1.56	264.12 ± 10.05	313.39 ± 11.90	58.23 ± 2.22
Artificial sebum-N	72.96 ± 4.51	1.97 ± 0.12	62.6 ± 3.87	101.67 ± 10.20	10.39 ± 1.04	18.26 ± 1.83	236.41 ± 11.77	280.52 ± 13.97	36.90 ± 1.84
Artificial sebum-S	5.68 ± 0.51	0.15 ± 0.01	2.63 ± 0.24	3.52 ± 0.41	0.36 ± 0.04	1.08 ± 0.12	3.62 ± 0.97	4.29 ± 1.15	2.18 ± 0.58
Artificial sebum-F	345.68 ± 7.78	9.19 ± 0.02	218.44 ± 0.56	488.73 ± 20.52	51.01 ± 2.46	86.90 ± 4.20	486.31 ± 14.88	586.39 ± 14.97	69.37 ± 1.77
Hamster body sebum	6.61 ± 1.13	0.15 ± 0.02	3.06 ± 0.42	8.76 ± 1.23	0.89 ± 0.13	1.43 ± 0.20	6.61 ± 1.13	7.85 ± 1.34	1.77 ± 0.30
Hamster ear sebum	75.73 ± 7.10	2.05 ± 0.02	42.20 ± 4.82	104.17 ± 4.47	10.65 ± 0.46	17.01 ± 0.73	104.53 ± 14.02	124.00 ± 16.60	28.02 ± 3.76

[a] Steady state flux through the artificial sebum.
[b] Permeability coefficient.
[c] Diffusion coefficient.

model compounds in hamster ear and body sebum samples, the sebum permeation properties (J_{ss}, P_s and D_s) of the compounds in hamster ear sebum are significantly different ($P > 0.05$) from that of hamster body sebum. This could be due to the differences in chemical composition of hamster body and ear sebum, and/or extraction of skin lipids or body lipids during the collection of hamster body sebum where the whole body was immersed in organic solvent to extract the body sebum. Moreover, the physical state of the sebum samples at 37 °C may play a critical role in governing the diffusion rate of the tested compounds. As shown in Table 4, hamster ear sebum liquefies at 35 °C while the body sebum melts completely up to 39 °C. The diffusion of molecules is faster through a liquid phase than through a waxy phase. The results of sebum diffusion studies demonstrate that none of the artificial sebum samples provides identical diffusion properties to the human sebum sample for all the model compounds, indicating that the kinetic factor of drug transport is much more sensitive to the small changes of the sebum chemical composition compared to the thermodynamic properties such as partition coefficient. However, based on the overall results, the diffusion properties of the model compounds in artificial sebum L are relatively closer to human sebum when compared with other artificial sebum and hamster sebum samples.

4. Conclusions

An artificial sebum (L) was designed based on the chemical compositions and thermal property of human sebum as well as the commercially availability of excipients. Artificial sebum L has similar DSC and NMR profiles to human sebum and provides an excellent alternative artificial sebum for drug partition and diffusion studies. Hamster sebum, either from the ear or the body, appears to be different from human sebum. The hamster body sebum is not recommended as a model for drug transport study because of its substantial deviation from human sebum.

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